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TITLE:

Induction and Acceleration of Mammary Tumors by Activated P13 Kinase

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14. ABSTRACT During this time period the necessary retroviral vectors were developed, used to transduce mammary epithelial cells, and these transduced cells were transferred into the cleared fat pad of recipient mice. Although ductal outgrowth occurred in the cleared fat pads indicating that the transplant was successful, the number of GFP positive ducts that grew out was very low. This was surprising given that a high proportion (greater than 80%) of the mammary epithelial cells were GFP-positive after infection. It is not clear whether the engraftment of the transduced cells was poor, or the outgrowth of these cells was diminished due to high expression of the transgene. Further studies related to these points were curtailed due to the departure of the graduate student from graduate school.					
15. SUBJECT TERMS Phosphatidylinositol 3'-kinase, mutation, breast cancer, mouse models					
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*Statement of Work***Training Plan:**

- Attend weekly journal club—DONE
- Attend weekly seminars—DONE
- Participate in AACR's pathobiology Cancer Workshop—NOT COMPLETED
- Attend PPG monthly meetings and retreat—DONE
- Present research at national/international research conferences—NOT COMPLETED

Research Plan:

Task 1: Determine whether the activating mutants of PI3 Kinase induce mammary tumorigenesis *in vivo*. (Months 1-18)

- a. Obtain PI3K mutant clones—DONE
- b. Insert PI3K mutants into pMIG vectors—DONE
- c. Obtain Bosc23 cells and pCL-Eco plasmid—DONE
- d. Infect Bosc23 cells with wildtype and mutant PI3K, and vector alone—DONE
- e. Collect supernatant containing infectious virus and filter—DONE
- f. Harvest primary MECs—DONE
- g. Infect and Transplant MECs encoding PI3K mutants, wildtype PI3K, and vector alone controls into recipient mice—DONE
- h. Optimize retroviral infection and transplantation protocols—DONE IN PART
- i. Evaluate mammary gland repopulation and ductal formation—NOT COMPLETED
- j. Determine phenotype of transplanted MECs infected with wildtype PI3K—NOT COMPLETED
- k. Determine phenotype of transplanted MECs infected with mutant PI3K—NOT COMPLETED
- l. Analyze tumors, if found, for protein expression levels—NOT COMPLETED
- m. Quantitate proliferation and apoptosis of tumors—NOT COMPLETED
- n. Analyze activation of signaling molecules downstream of PI3K—NOT COMPLETED

Task 2: Determine whether the activating mutants of PI3 Kinase accelerate mammary tumorigenesis induced by HER2/Neu *in vivo*. (Months 12-30)

- a. Infect Bosc23 cells with wildtype and mutant PI3K, and vector alone—DONE
- b. Collect supernatant containing infectious virus and filter—DONE
- c. Obtain MECs from MMTV-c-Neu mice—NOT COMPLETED
- d. Infect and Transplant MECs with PI3K mutants, wildtype PI3K, and vector alone controls—NOT COMPLETED
- e. Visualize for successful infection of MECs—NOT COMPLETED
- f. Evaluate mammary gland repopulation and ductal formation—NOT COMPLETED
- g. Evaluate kinetics of tumor formation—NOT COMPLETED
- h. Analyze tumors for differences in histology, protein expression, and metabolism when compared to MMTV-c-Neu control tumors—NOT COMPLETED
- i. Quantitate proliferation and apoptosis of tumors—NOT COMPLETED
- j. Analyze activation of signaling molecules downstream of PI3K and c-Neu—NOT COMPLETED

Task 3: Determine if the effects of activated PI3 Kinase on mammary tumorigenesis are dependent upon Akt. (Months 20-36)

- a. Infect Bosc23 cells with wildtype PI3K and c-Neu*—NOT COMPLETED
- b. Infect Bosc23 cells with mutated PI3K and c-Neu*—NOT COMPLETED
- c. Infect Bosc23 cells with vector alone—NOT COMPLETED
- d. Collect supernatant containing infectious virus and filter—NOT COMPLETED
- e. Obtain MECs from Akt 1 KO mice—NOT COMPLETED

- f. Obtain MECs from FVB mice (Akt +/+)—NOT COMPLETED
- g. Infect MECs (Akt KO and Akt +/+) with collected virus—NOT COMPLETED
- a. Transplant infected MECs into recipient mice—NOT COMPLETED
- b. Evaluate mammary gland repopulation and ductal formation—NOT COMPLETED
- c. Monitor mice for health and tumor growth—NOT COMPLETED
- d. If tumors are found; analyze protein expression levels—NOT COMPLETED
- e. Quantitate proliferation and apoptosis of tumors, if found—NOT COMPLETED
- f. Analyze activation of signaling molecules downstream of PI3K and c-Neu*—NOT COMPLETED

*If PI3K is unable to form tumors without the addition of c-Neu